The Value of Space Research for the Biotechnology and Pharmaceutical Industry

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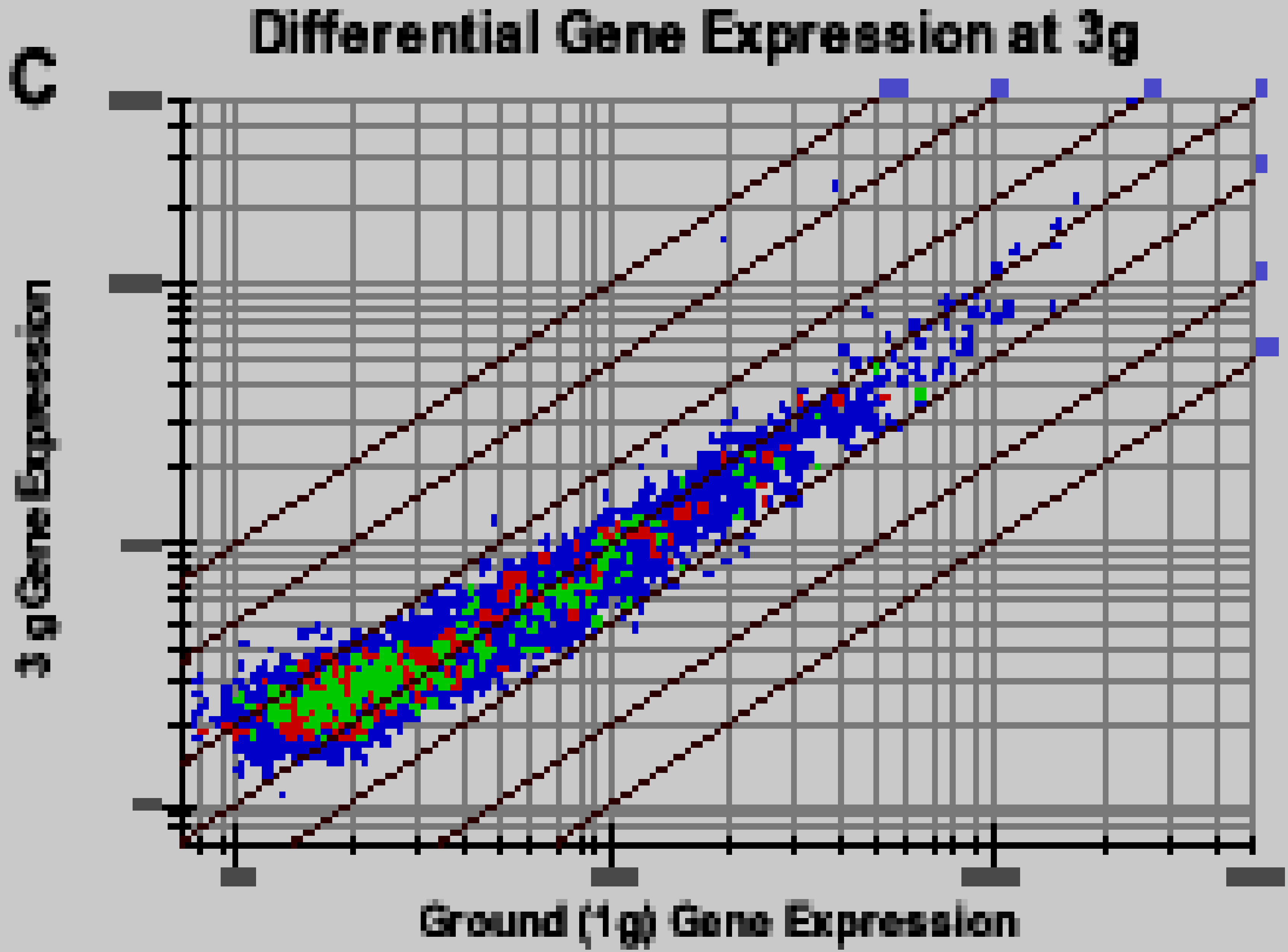
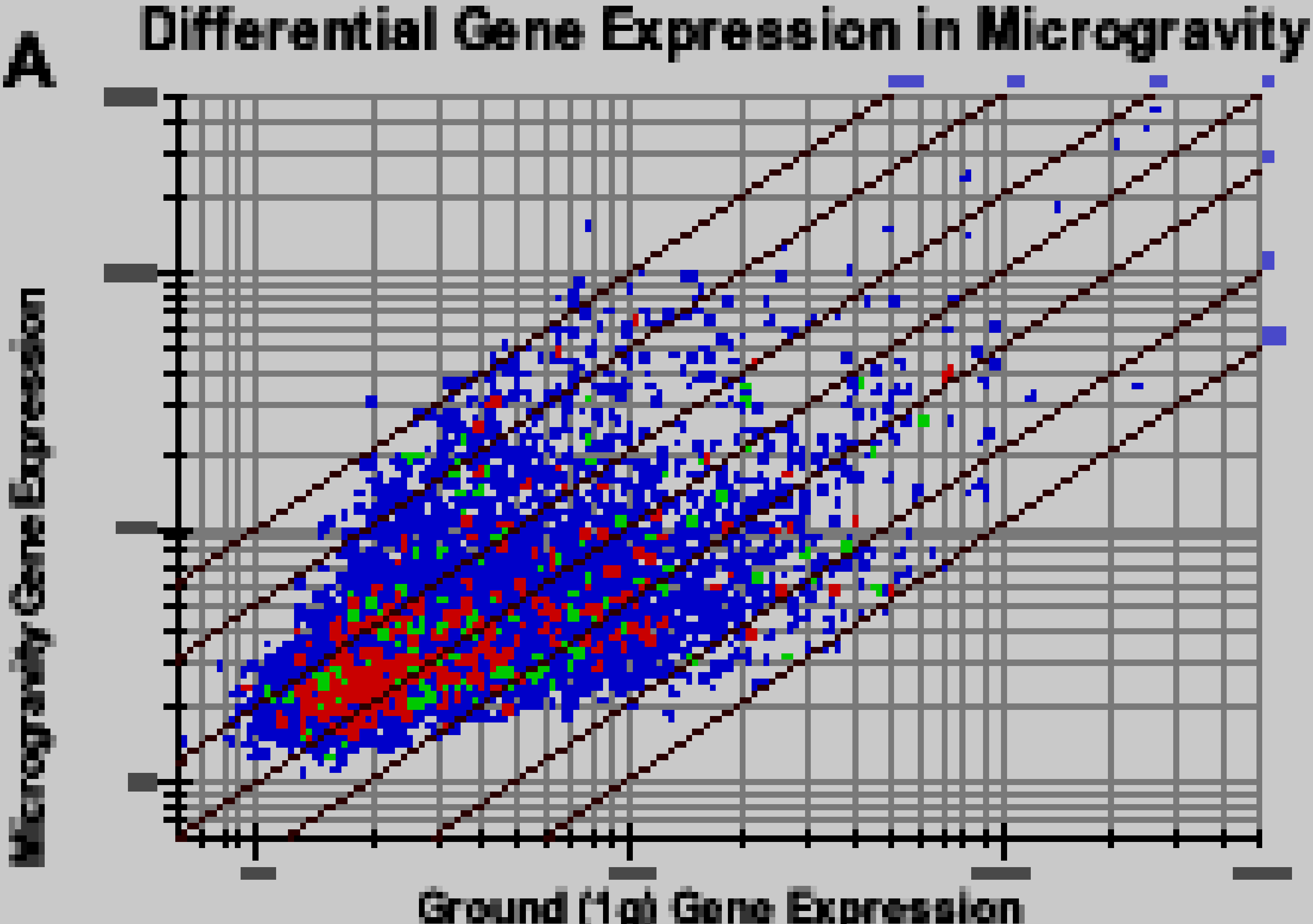
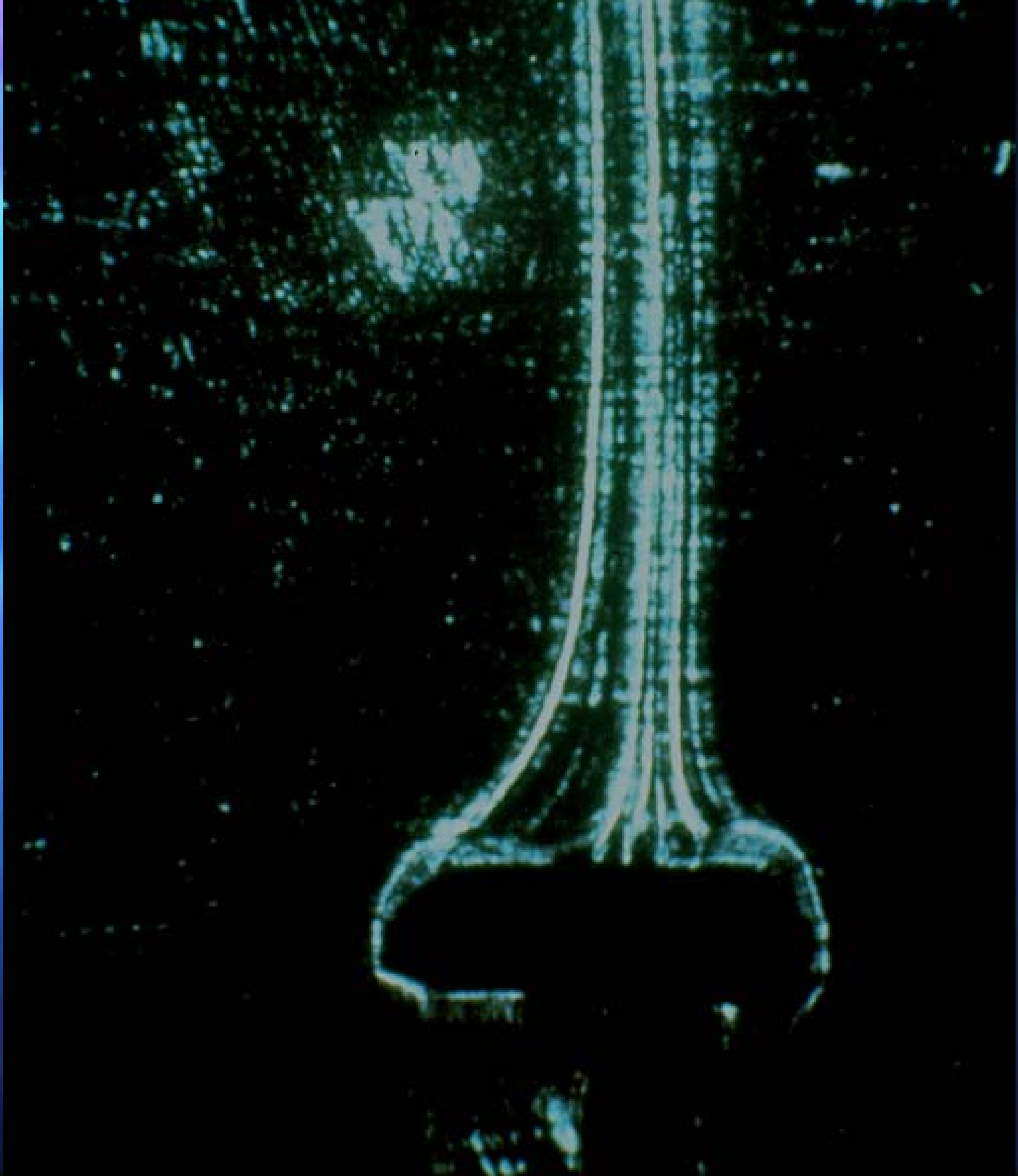
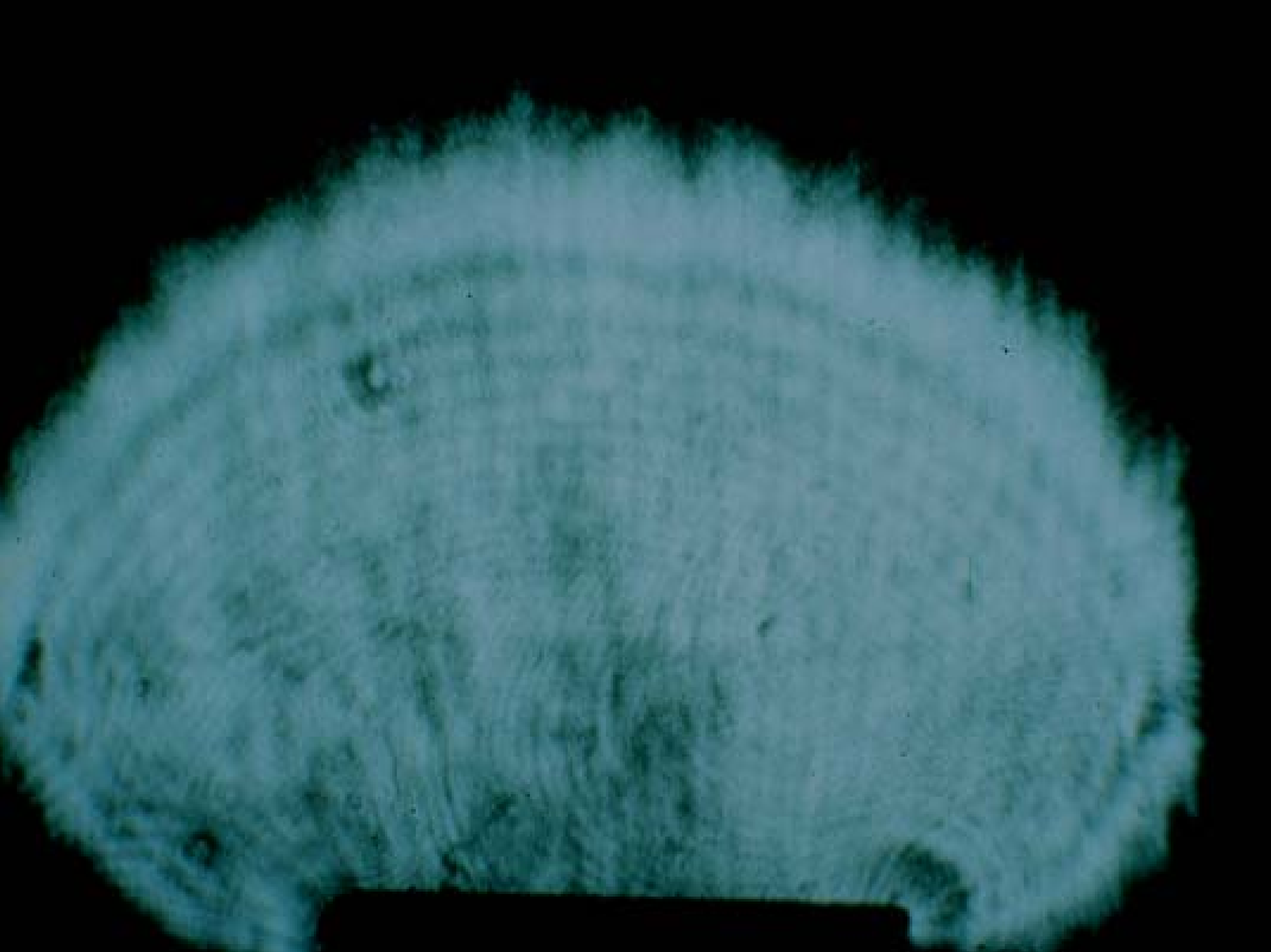

Differential Gene Expression at 3g
C
3 g Gene Expression
Ground (1g) Gene Expression



A
Differential Gene Expression in Microgravity
Microgravity Gene Expression
Ground (1g) Gene Expression



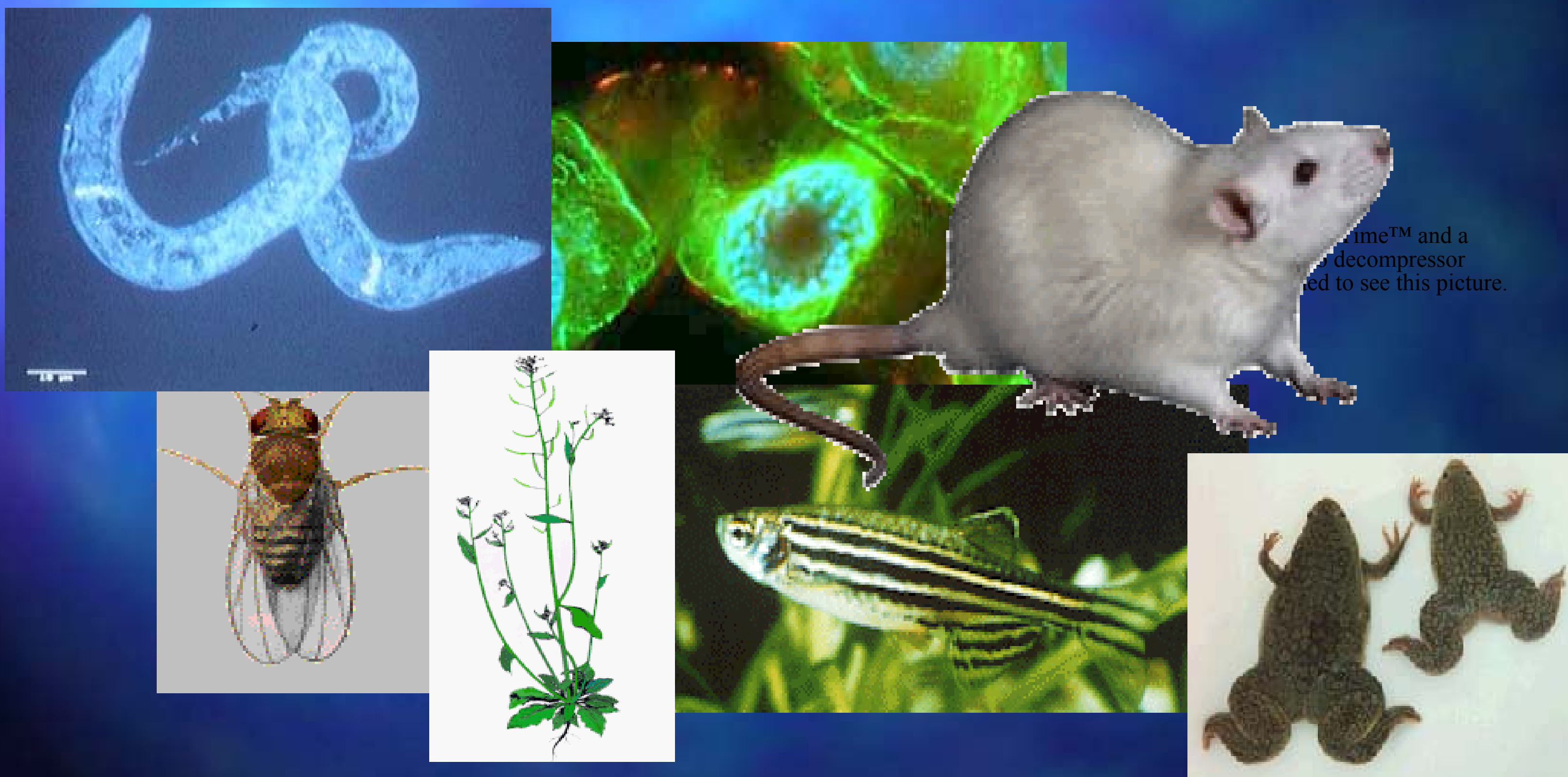


Fundamental Space Biology Program

- Study of fundamental biological processes through space flight and ground-based research. The program sponsors research into the affects of space on the evolution, development, and function of living organisms.
- Scientific Research Elements
  - Molecular Structures and Physical Interactions
  - Molecular Biology
  - Cell Biology
  - Organismal and Comparative Biology
  - Developmental Biology
  - Evolutionary Biology
  - Gravitational Ecology

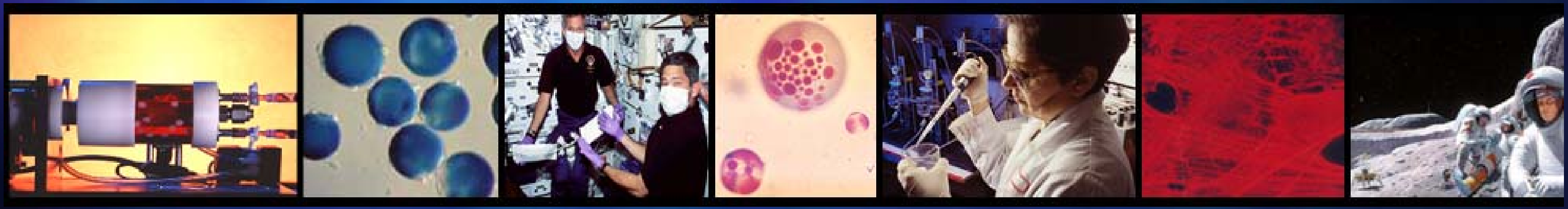
- Revolution technology enhancements have radically changed the world of biological research. As a result, technology now plays a central role in accomplishing scientific goals.
  - In-Situ Measurement and Control
  - Genomics/Proteomics Technologies
  - Biological Information Processing
  - Bioimaging Systems

in-situ TECHNOLOGIES for FUNDAMENTAL BIOLOGY RESEARCH



Cellular Biotechnology Objectives

- Improve human health on earth by application of NASA-developed technology in innovative research endeavors
- Facilitate basic and applied research of peer-reviewed science in cell biology and tissue engineering
- Contribute to space exploration by providing technological advances in life support, health care, and space research
- Provide ground-based and space cell culture technology for the science community requiring microgravity and altered gravity environments to achieve scientific goals
  - Optimize use of laboratory facilities on International Space Station
- Promote space cell biology as an academic discipline



Along the way …

Cellular Biotech bioreactor-based research has resulted in numerous 'Firsts'!

Year	Bioreactor Accomplishments – *First to Ever…..*
1992	• Develop a three dimensional co-culture process
1995	•• Culture high quality ovarian cancer tumors for cancer research •• Grow cartilage cells on biodegradable scaffolds
1996	•• Culture normal mammalian tissue ▪• Produce Taxane in haploid-derived cell cultures
1997	•• Transplant Sertoli cells as neurorecovery inducing cells for Parkinson's disease •• Perform recombinant production of selected polypeptides using transformed insect cells
1998	•• Produce a sustained localized brain immunosuppressive effect in localized tissues •• Grow human renal tubular cells which formed large tissue aggregates •• Develop *in vitro* system to permit study of HIV in human lymphoid tissue on Earth •• Co-culture endothelial cells and human breast carcinoma •• Culture 3D human urogenital tract carcinomas
1999	•• Produce three-dimensional optic tissue •• Produce normal mammalian organ culture •• Develop model for metastasis of colon cancer cells to the liver •• Develop cell culture models of immune dysfunction using human tonsilar tissue •• Grow cell cultures of endangered cetacean (bowhead whale) kidney •• Demonstrate unique glycosylations in insect cells expressing human genes
2000	•• Demonstrate coordinated beating of engineered cardiac tissue, showing spontaneous contractions at a rate of 45 beats per minute •• Develop a process for propagating a pathogen in a 3D tissue mass
2002	•• Produce 3D tissue-like aggregates of muscle cells from solid fat •• Direct nerve cell growth by stimulating gene sequences by time varying electromagnetic field
2003	•• Produce the largest co-culture of prostate cancer/bone cells

Applications to Human Health Issues on Earth and ISS…

CANCER (breast, skin, prostate, ovary, bone and colon)	▪ Helping understand cancer growth and human immune system response ▪ Testing three-dimensional tissues for sensitivity to chemotherapy and hormonal therapy
DIABETES	▪ Working on pancreatic tissue for transplant ▪ Working on bioreactor research to understand the best route for cultivating and transplanting beta cells into Type I diabetics
HEART DISEASE	▪ Using bioreactor to find results for heart muscle research, drug testing, and the possibly eventual growth of transplantable heart tissue ▪ Studying how heart cells interact to form cardiac structures outside the body
BONE LOSS	▪ Studying the mechanisms involved in bone loss related to microgravity ▪ Using analog microgravity cultures to determine the optimal characteristics of tiny glass particles for growing bone tissue
INFECTIOUS DISEASES	▪ Establishing the NASA/NIH Center for Three Dimensional Tissue Culture which provides researchers an opportunity to develop new model systems for diseases whose pathology cannot be reproduced by merely growing the right cells in monolayer culture. ▪ Human immunodeficiency virus (HIV) ▪ Ebola virus – Using the bioreactor to grow cultures—rather than using live animals – in the study of how the Ebola virus is transmitted. ▪ Lyme disease virus – Studying Borrelia, the etiologic agent of Lyme disease ▪ Model for Salmonella pathology in engineered colon tissue
ACUTE INJURY AND RECONSTRUCTIVE SURGERY	▪ Using bioreactor to grow cartilage cells on biodegradable scaffolds ▪ Using the International Space Station to produce models and transplantable cartilage tissues that could revolutionize treatment for joint diseases and injuries ▪ Investigation of cytokine synthesis in analog culture to address wound healing
DRUG EFFICACY	▪ Facilitating testing of drugs to determine their safety without using animals and reducing the need to use human volunteers in final testing ▪ Supporting space research on an enzyme that HIV needs to reproduce to define the enzyme's structure so that effective pharmaceuticals can be developed to inhibit the HIV virus

Cellular Biotechnology Research: Countermeasures

Why cellular research for countermeasures development?

- Basis for Life
- Responses of transitioning life to microgravity are based at the cellular level in all organisms
- Investigations are conducted at the cellular level in an attempt to elucidate the basis of the human response to microgravity.
  - Understanding cellular-level responses is necessary to explain and control human adaptation to space critical to the exploration and development of space.
- Shorter generation times of bacteria and human cells provide early inroads to a better understanding of adaptation of life to the space environment

Cellular Biotechnology Research Questions

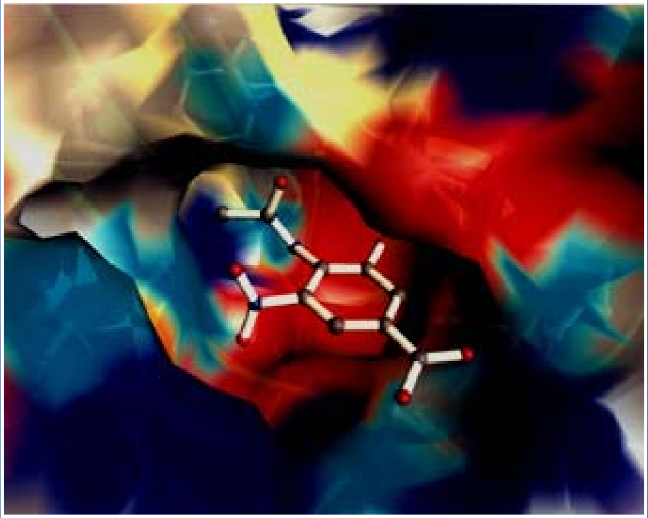
- Adaptive responses of cells to microgravity and the space environment?
  - Shape and Orientation of subcellular components
  - Signal transduction, cell division, and gene expression
  - DNA damage and cellular repair
  - Programmed cell death and cellular movement
  - Cytoskeleton redistribution
  - Loss of polarization and movement
  - Functional changes like movement and differentiation
- Phenotypic and genotypic changes induced by microgravity, space, and planetary environments?
- Does the space environment invoke a selective pressure on replicating cells?
- Do the core cellular metabolic systems change in space?
- What is the impact on higher cellular orders tissues, organs, systems, and organisms?

QuickTime™ and a
Sorenson Video decompressor
are needed to see this picture.


Center for
Biophysical Sciences and Engineering

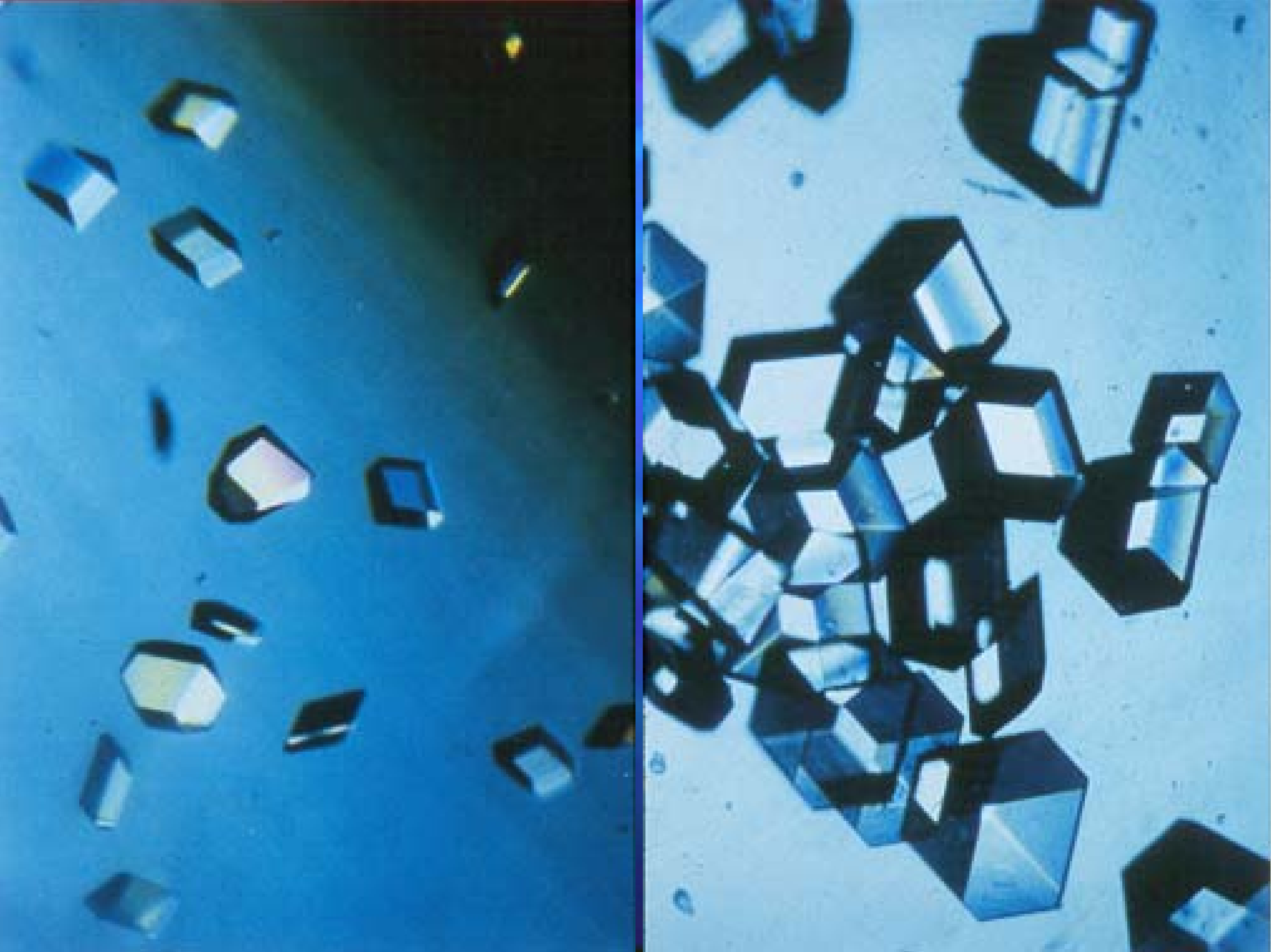
CBSE Science Capabilities

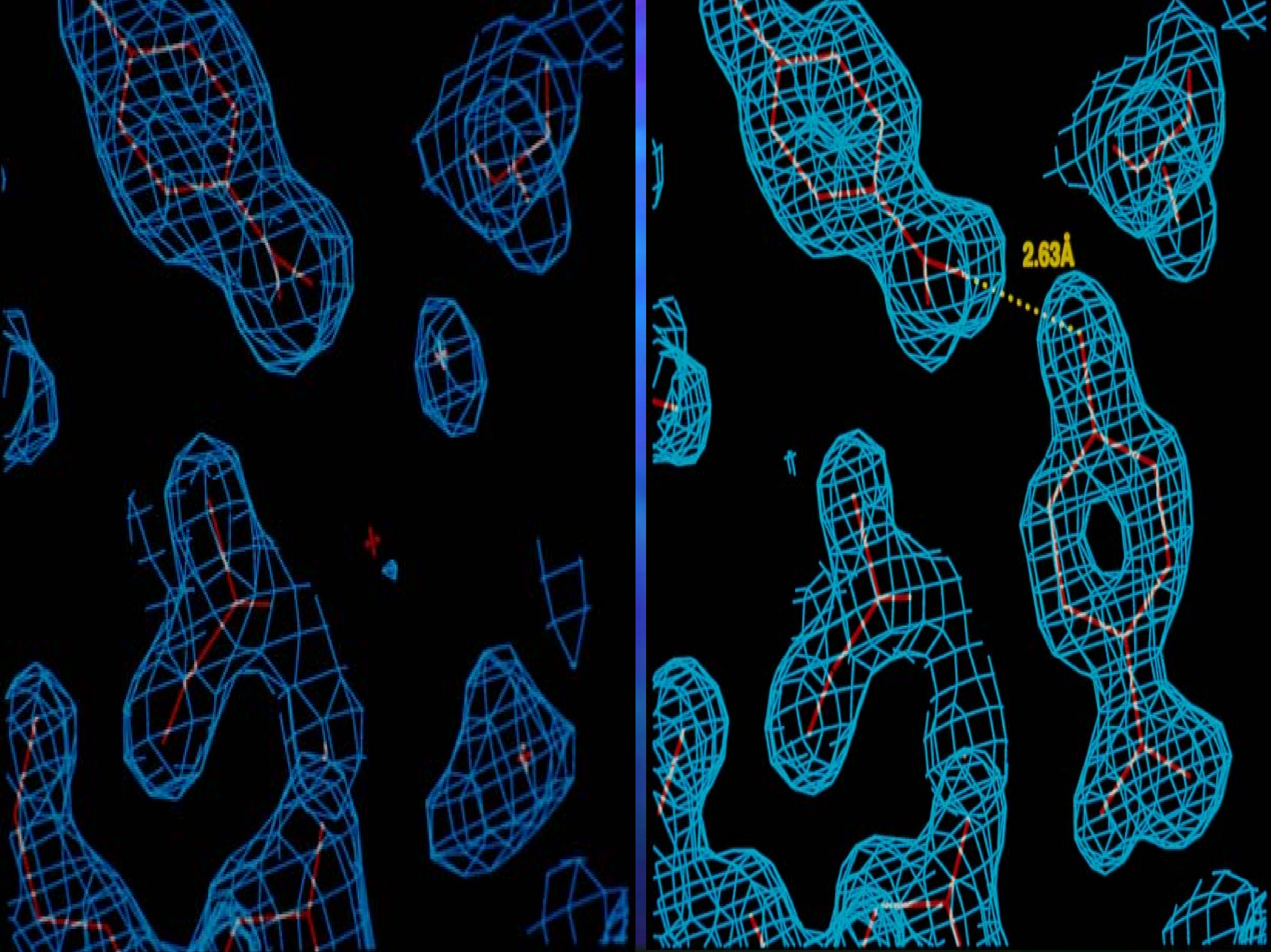
- Cloning & Expression
- Protein Purification
- Crystal Growth
- Structure Determination
- Structure-Based Drug Design
- Structural Energetics
- Medicinal Chemistry
- Structure-Directed Combinatorial Chemistry
- High-Throughput Screening
- Assay Development
- Preclinical Trials (*in-vitro* and *in-vivo*)



CBSE Engineering Capabilities

- Engineering (51 Aerospace Engineers):
  - Design
  - Mechanical
  - Stress Analysis
  - Software
  - Electrical
  - NASA Documentation/
  - Certification and Testing
  - Precision Machine Shop



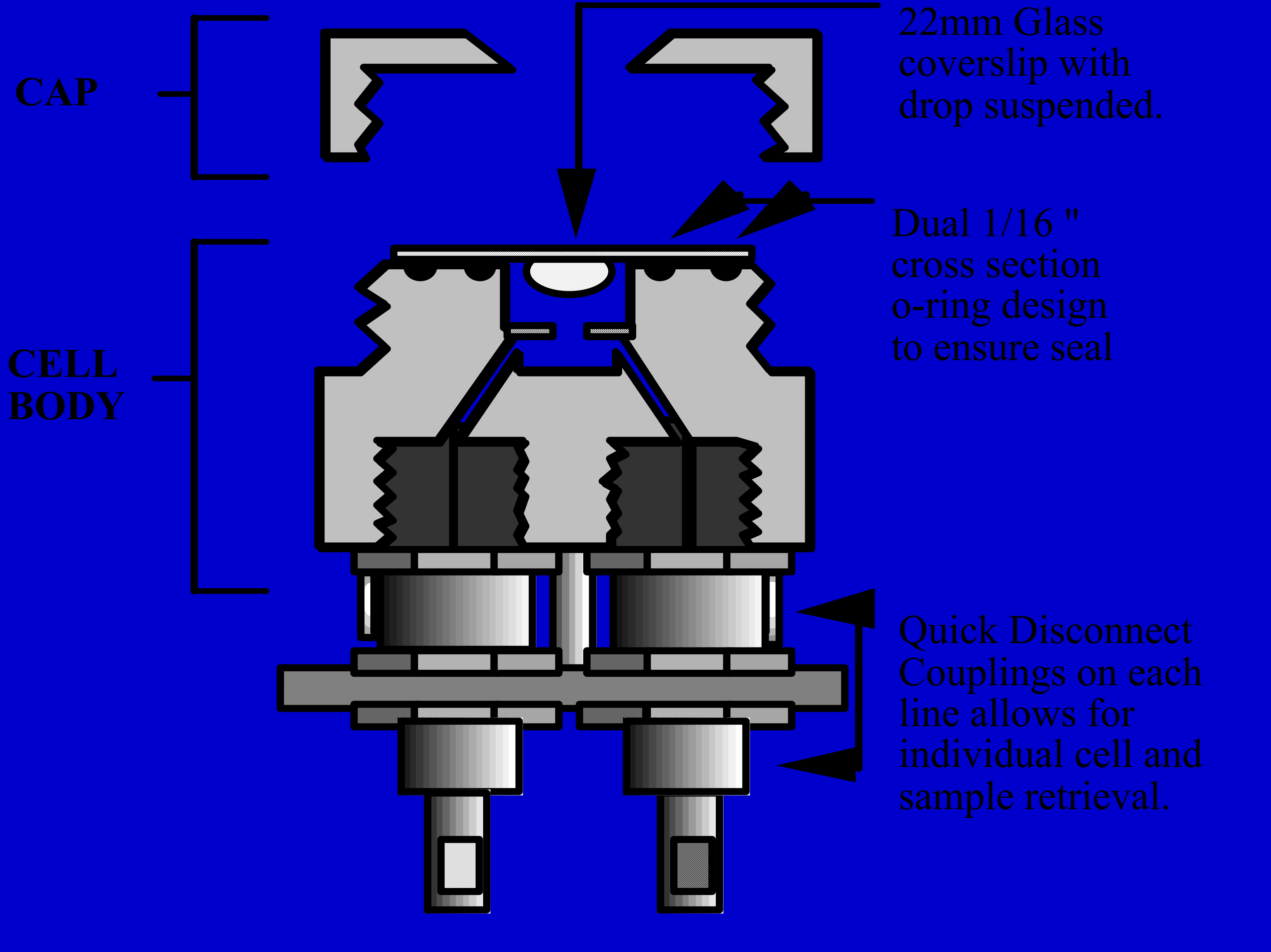

2.63Å

NIH Structural Genomics Initiative: Summary of Crystallization Results

	Soluble Proteins	Crystallized	Diffraction Quality	Structures
Total Number	7,529	2,294	1,050	438
Percentage		30.4 %	13.9 %	5.8 %

Technology Development

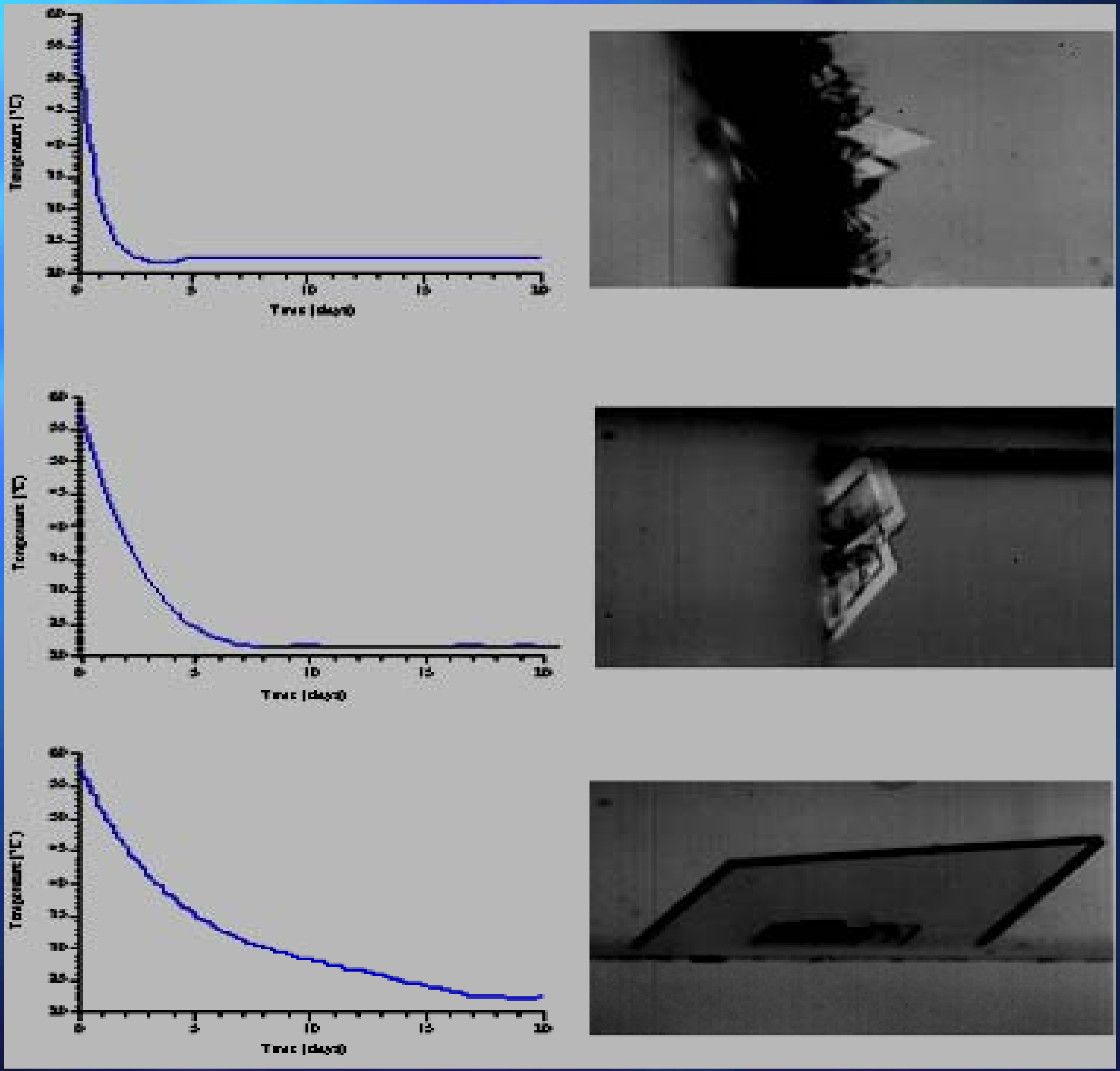
- Dynamic Control of Protein Crystallization
- Nano-crystallization and automated image analysis
- Predictive algorhythms provide the ability to perform virtual screens of crystallization space
- Microfluidics applied to crystallization


CAP
CELL
BODY
22mm Glass
coverslip with
drop suspended.
Dual 1/16 "
cross section
o-ring design
to ensure seal
Quick Disconnect
Couplings on each
line allows for
individual cell and
sample retrieval.

VaporPro

Automated image acquisition and control of vapor diffusion experiments for optimization

Dynamically Controlled Crystallization Results




1. 23. 2003

"ScreenPro"
A Self-Learning Crystallization System

- Neural net uses limited data to predict new crystallization conditions
  - Reduces screen size
  - May find crystallization trends/families

Comparison of Experimental Conditions that Yield Crystal for Delta 8-10B

Experim ent	ture	[Protein] dilution	[buffer] buffer	pH	[salt] salt	organic, %	Organic	[Divalent] Divalent	[Additiv e] Additive	SCORE
Trained	14	2.300	0.100 M Bicine	8.3	0.567 M Na Acetate	0.9 % PEG400	0.0	0.010 M $CaCl_2$	0.050 % BOG	6
Predicted	22	1.500	0.100 M Acetate	4.5	0.648 M Na Chloride	11.6 % PEG M5000	0.0	0.010 M $CaCl_2$	0.000 None	6

Comparison of the only crystal in the training set to the only crystal in the test set. The two crystal conditions are fairly disparate.

Diversified Scientific, Inc.

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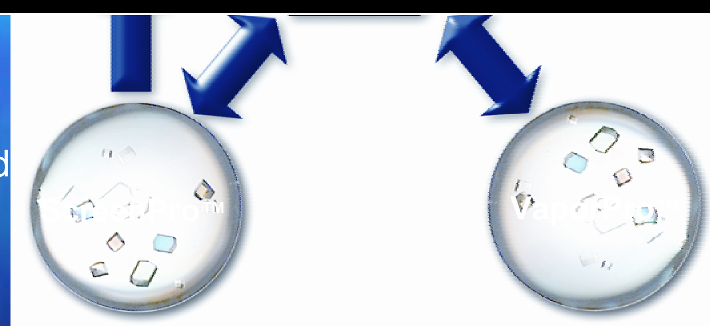
Intelligent design tool for crystallization robots

Develop optimizations based upon incomplete factorial sampling algorithms, grid screen algorithms, or user-specified, custom conditions

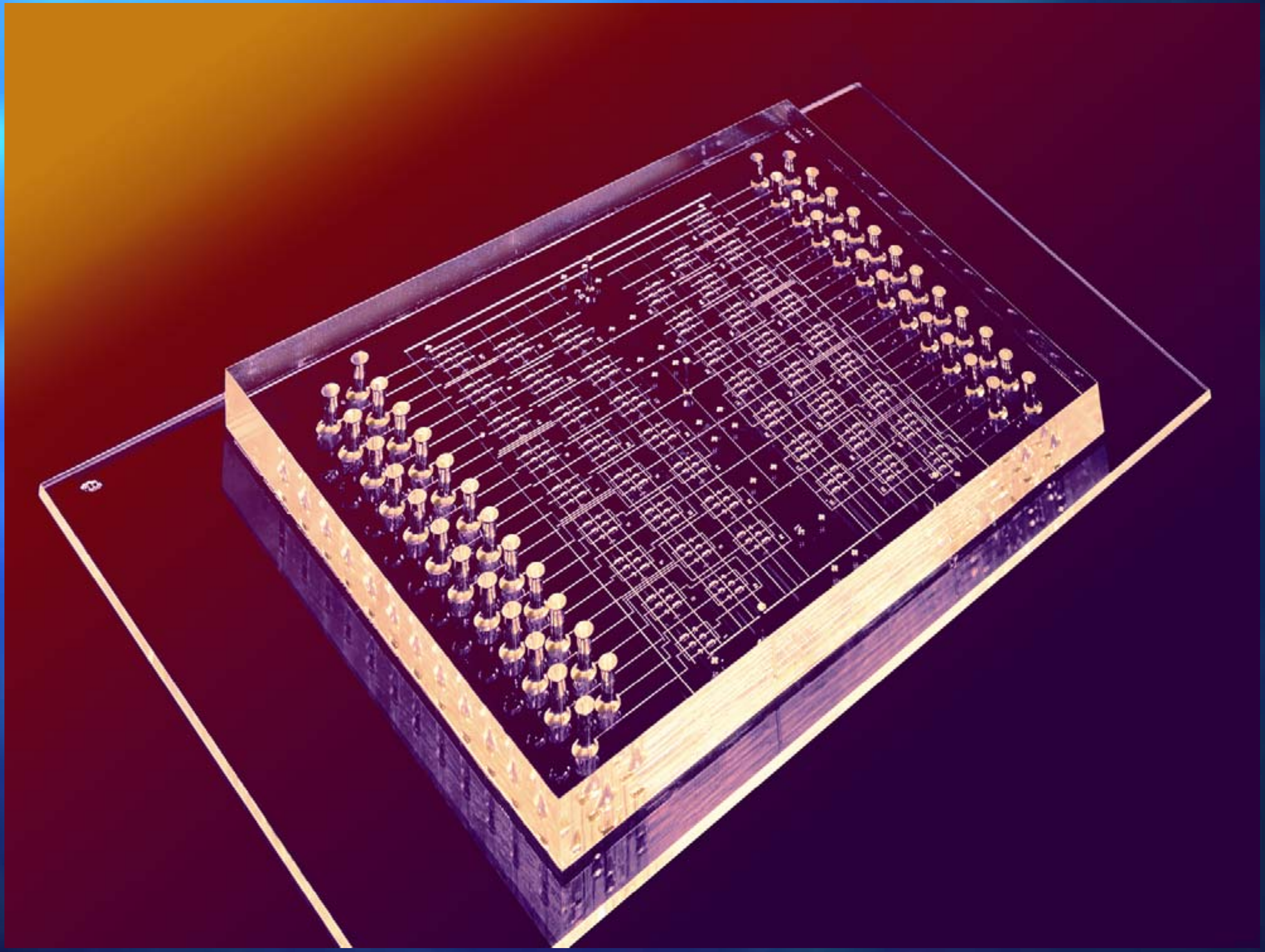


Allows for slow progression to supersaturation, nucleation, and decreased growth rate

Build optimization screens derived from all outcomes, including failures with neural network technology

Fluidigm™

The Topaz™ Crystallization Microprocessor



The Topaz™ Crystallizer for Protein Crystallization

Topaz Microprocessor

Fluid Control Assembly

Microprocessor Carrier